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The Bench-to-Bedside Transition

Acute metabolic acidosis in a GLUT2-deficient patient with Fanconi–Bickel syndrome: new pathophysiology insights

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ABSTRACT

Fanconi–Bickel syndrome is a rare autosomal-recessive disorder caused by mutations in the *SLC2A2* gene coding for the glucose transporter protein 2 (GLUT2). Major manifestations include hepatomegaly, glucose intolerance, post-prandial hypoglycaemia and renal disease that usually presents as proximal tubular acidosis associated with proximal tubule dysfunction (renal Fanconi syndrome). We report a patient harbouring a homozygous mutation of *SLC2A2* who presented a dramatic exacerbation of metabolic acidosis in the context of a viral infection, owing to both ketosis and major urinary bicarbonate loss. The kidney biopsy revealed nuclear and cytoplasmic accumulation of glycogen in proximal tubule cells, a lack of expression of GLUT2, and major defects of key proteins of the proximal tubule such as megalin, cubilin and the B2 subunit of H^+–ATPase. These profound alterations of the transport systems most likely contributed to proximal tubule alterations and profound bicarbonate loss.

Keywords: Fanconi–Bickel, ketosic acidosis, metabolic acidosis, proximal tubular nephropathy, *SLC12A2*

INTRODUCTION

Fanconi–Bickel syndrome is a rare autosomal-recessive disorder caused by mutations in the *SLC2A2* gene that codes for the glucose transporter protein 2 (GLUT2). Because this transporter is expressed in the liver and pancreas (β-cells), intestine and kidney, patients typically show hepatomegaly due to glycogen accumulation, glucose intolerance, post-prandial hypoglycaemia and proximal tubulopathy. The disease was first described by Fanconi and Bickel in 1949 in a 3-year-old Swiss boy [1]. To date, >200 patients have been reported, and about 34 different mutations of *SLC2A2* have been identified [2]. The renal disease usually presents as a moderate proximal tubular acidosis associated with generalized proximal tubule dysfunction (renal Fanconi syndrome). Kidney biopsies are rarely performed, and the mechanisms whereby *SLC2A2* mutations induce proximal tubule dysfunction are essentially unknown.

In this brief report, we describe the case of a patient harbouring a *SLC2A2* mutation presenting with acute exacerbation of acidosis compatible with superimposed acute tubular necrosis. Immunohistochemical studies of the kidney biopsy showed profound alterations in the expression of major proteins sustaining reabsorptive functions of the proximal tubule.

CASE REPORT

The patient, of Algerian origin, was diagnosed in 1976 at the age of 19 months with a Toni-Debré-Fanconi syndrome, consisting of hyperphosphaturia, glycosuria, aminoaciduria, moderate metabolic acidosis and hypercalciuria. The combination of severe growth retardation, hepatomegaly and hypoglycaemia led to the diagnosis of Fanconi–Bickel syndrome when the patient was 6 years old. Genetic analysis showed a homozygous mutation (IVS 3+2t>c/IVS 3+2t>c) in the *SLC2A2* gene

[3]. This splice site mutation produces an irregular amino acid sequence with a premature termination of translation at the 44th codon after the lost splice site [3]. Although the parents claimed no consanguinity, grandparent inbreeding was suspected because they were native from the same village. A first kidney biopsy, performed at the age of 5, found glycogen deposits in proximal tubules (Figure 1Aa). Therapy was initiated (treatment doses from 2000 onwards) with vitamin D (calcitriol 0.5 µg/day), potassium gluconate (syrup 15% 30 mL/day) and phosphate supplementation (concentrated phosphoric acid, 450 drops/day). Serum creatinine was equilibrated at 70 µmol/L, HCO_3 at 25 mmol/L, K at 3.6 mmol/L and PO_4 at 0.6 mmol/L (Table 1).

In 2011, at the age of 36, the patient (height 1.44 m, body mass index 18) was admitted to the intensive care unit because of hyperthermia caused by serologically proven myxovirus influenzae A. Investigations (Table 1) revealed severe metabolic acidosis (HCO_3, 5 mmol/L) with a profound serum anion gap (36 mmol/L) due to severe ketosis (ketonaemia, 6.9 mmol/L) with ketonuria (3+). Glycaemia (6 mmol/L) and serum lactate levels (1.7 mmol/L) were normal. Renal function was stable (serum creatinine 62 µmol/L and $eGFR_{MDRD}$ 93 mL/min/1.73 m^2).

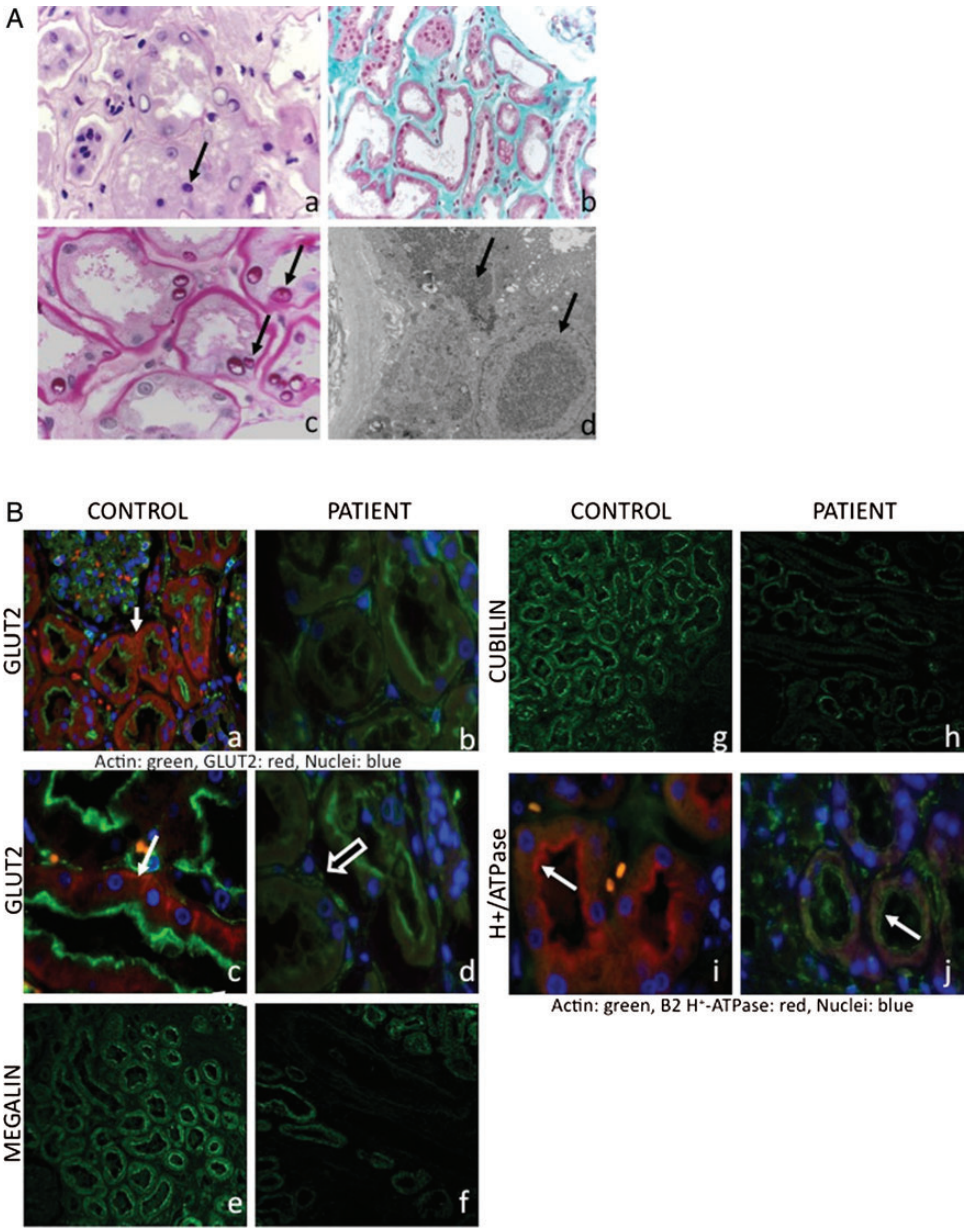

FIGURE 1: (**A**) (a) First kidney biopsy at the age of 5 (1980), periodic acid–Schiff staining showing nuclear and cytoplasmic glycogen inclusions. (b–d) Second kidney biopsy (2011). The trichrome staining shows foci of acute tubular necrosis and proximal tubular lesions (b). Nuclear and cytoplasmic glycogen inclusions are seen by periodic acid–Schiff stain (c, arrow) and by electron microscopy (d, arrow). (**B**) Immunohistochemistry for GLUT2, megalin, cubilin and B2 H^+–ATPase subunits showing normal localization on proximal tubular cells in control kidney and the lack of expression in the patient. GLUT2 and B2 H^+–ATPase subunits were stained in red whereas actin, megalin/cubilin, was stained in green and nuclei in blue.

Table 1. Serum and urinary data before, during the two steps of the exacerbation and after the event

	Before event (2007–32 years old)	During event		After event (29 June 2013–38 years old)
		Ketosic decompensation (6 January 2011–36 years old)	Proximal tubule defect (12 January 2011–36 years old)	
Serum creatinine (μmol/L)	60	62	52	67
HCO_3 (mmol/L)	24	5	8	23
Cl (mmol/L)	105	106	115	102
Serum anion gap (mmol/L)	–	36	12	–
Ca (mmol/L)	2.54	2.22	1.88	2.29
Albuminaemia (g/L)	52	31.7	22.9	39.9
PO_4 (mmol/L)	0.46	0.7	0.8	0.7
Ketonaemia (mmol/L)	ND	6.9	<0.1	ND
Urinary anion gap (mmol/L)	48	54	125	33
Ketonuria	0	+++	0	0
Urinary RBP (mg/mmolC)	11	ND	20	12.8
Urinary albumin (mg/mmolC)	56	ND	57	21
25-OH vitamin D_3 (ng/mL, 30–100)	20	ND	ND	12
1,25-$(OH)_2$ vitamin D_3 (pg/mL, 17–67)	69	ND	ND	56.4

RBP, retinol binding protein; +++, ketonuria assessed by urinary dipstick on a scale from 0 to +++; ND, not determined.

The patient was rehydrated with sodium bicarbonate 1.4% and placed under respiratory support because of polypnoea and exhaustion. Insulin therapy was started. The serum anion gap returned to the normal range 12 mmol/L, but severe metabolic acidosis of renal origin persisted, with a positive urinary anion gap (125 mmol/L), low ammoniuria level (1 mmol/L), high bicarbonaturia (55 mmol/L) and urinary pH (7.55). The blood–urine pCO_2 gradient was calculated at 40 mmHg, which excluded distal tubular involvement. Molar bicarbonate infusion was started and tubular dysfunction progressively improved. Finally, electrolytes returned to baseline levels (Table 1). Bone densitometry revealed a *T*-score of −3.3.

A second kidney biopsy was performed because of persisting severe renal acidosis. The biopsy revealed severe lesions with acute tubular necrosis with loss of brush border membranes and nuclear and cytoplasmic inclusions compatible with glycogen deposits in proximal tubules (Figure 1Ab–d). Glomerular and interstitial compartments were normal. Electron microscopy confirmed glycogen accumulation (Figure 1Ad). Immunoreactivity for GLUT2 (AbDSerotec, Kidlington, UK), megalin and cubilin [4] and H^+–ATPase pump (subunit B2) [5] was investigated by immunofluorescence staining, in comparison with control kidneys. Figure 1B shows the basolateral localization of GLUT2 in the human early proximal tubule of control kidneys and the absence of this transporter in the patient. Figure 1B also shows dramatically reduced expression of megalin and cubilin and the lack of expression of the B2 H^+–ATPase subunit in the patient compared with control kidneys.

DISCUSSION

Fanconi–Bickel syndrome is a well-characterized clinical entity, which combines generalized proximal tubule dysfunction, impaired utilization of glucose and galactose, and hepatorenal glycogenosis. The diagnosis of Fanconi–Bickel syndrome was confirmed by the finding of a homozygous mutation (IVS 3+2t>c/IVS 3+2t>c) in *SLC2A2*, that predicts the synthesis of a truncated protein. To our knowledge, this mutation is unique to our patient.

The glomerular filtration rate was normal and stable in the patient, as described by others [6]. In contrast, his presentation included manifestations of severe proximal tubule dysfunction, with a severe, unusual metabolic acidosis.

Severe metabolic acidosis is exceptional in Fanconi–Bickel syndrome. In our patient, severe metabolic acidosis resulted from two mechanisms. First, the high anion gap metabolic acidosis observed initially was due to accumulation of ketone bodies induced by a major defect in glucose and galactose utilization aggravated by myxovirus influenzae A infection and stress. This defect is primarily caused both by the alteration of the GLUT2 transporter and by insulinopaenia resulting from expression of the mutated GLUT2 in pancreatic beta cells [7]. Notably, ketosis occurred without hyperglycaemia because of urinary glucose loss. The second component of metabolic acidosis was of renal origin and linked to the proximal tubule defect with hypophosphataemia, glycosuria and low-molecular-weight proteinuria. Acid–base balance was only sustained by large bicarbonate supplementation until recovery of tubular lesions.

Analysis of kidney biopsy provided new insights into the molecular mechanisms of the disease. First, it showed the lack of GLUT2 expression at the basolateral side of proximal tubule cells (compare Figure 1Bd with Figure 1Ba and Bc, arrow), which were filled with glycogen vacuoles. Secondly, immunohistochemistry showed highly reduced megalin and cubilin (compare Figure 1Bf and Bh with Figure 1Be and Bg, respectively), both required in tubular absorption of ligands and recycling of transporters through the endocytotic compartment [8], accounting for the high urinary loss of retinol binding protein. In addition, the B2 subunit of the H^+–ATPase involved in proximal tubule reabsorption of bicarbonate and megalin- and cubilin-dependent endocytosis of proteins was also lacking (compare Figure 1Bj with Figure 1Bi, arrow). Similar impaired expression of key molecules of proximal tubular function have been reported in mice and humans with genetic alterations in the hepatic nuclear factor 1 alpha

(HNF1α) and 4 (HNF4) transcription factors leading to a Fanconi syndrome-like phenotype [9, 10]. In our patient, these major phenotypic alterations were most likely induced by chronic accumulation of glycogen leading to cell injury. We hypothesize that these alterations account for the severe tubular dysfunction including renal acidosis observed in the Fanconi–Bickel syndrome. It is likely that, in the context of severe metabolic acidosis and superimposed tubular necrosis, these alterations were aggravated. Return of the renal parameters, including serum bicarbonate and urinary anion gap to baseline values, suggests that these additional lesions recovered, although they may influence the renal outcome later on.

In conclusion, we present a case of severe metabolic acidosis in a patient with Fanconi–Bickel syndrome which led to unravel at least some molecular bases for defective tubular transport.

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CONFLICT OF INTEREST STATEMENT

None declared.

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